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THE VERTICILLIUM DISEASE OF THE POTATO.

BY

GEORGE H. PETHYBRIDGE, PH.D., B.SC.,

ECONOMIC BOTANIST TO THE DEPARTMENT OF AGRICULTURE AND TECHNICAL INSTRUCTION FOR IRELAND.

(PLATES II–III.)

[*Authors alone are responsible for all opinions expressed in their Communications.*]

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VII.

THE VERTICILLIUM DISEASE OF THE POTATO.

By GEORGE H. PETHYBRIDGE, Ph.D., B.Sc.,

Economic Botanist to the Department of Agriculture and Technical Instruction for Ireland.

(Plates II–III.)

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I. Introductory.

During the past seven summers special investigations dealing with various diseases of the potato have been pursued in Ireland, where the potato crop is such an important one. Some of the diseases dealt with have been new, while others, although not new, were more or less obscure and had previously been insufficiently studied, so that it was possible to throw new light on them.

The results of these investigations have been published annually in the form of general summaries or reports, and in certain cases the diseases have formed the subjects of special scientific papers.[1]

The disease with which the present paper deals has been referred to briefly in these reports from 1910 onwards, but no detailed account of it has yet been given. It is not really a new disease, although up to the time of starting the present investigations comparatively little was known about it, and definite record of its presence in the British Isles had apparently not been made. It has, in fact, long remained incompletely studied and concealed amongst the congeries of diseases passing under the names of Curl and Leaf-Roll, from which, however, it must now be removed and be recognized as a distinct disease of a definite type caused by a specific parasitic organism, as will be clear from the discussion in Section VIII of this paper.

The reason why the present study of the disease has been a somewhat protracted one is partly because the disease was only one of several which

[1] These reports will be found in the Journal of the Department of Agriculture and Technical Instruction for Ireland, vols. x, xi, xii, xiii, xiv, and xv, 1910 to 1915. References to the special scientific papers mentioned will be found in these reports.

were being studied simultaneously, but more especially because it was found that in order to obtain accurate information upon the extent and mode of transmission of the disease by affected plants to their progeny it was necessary to extend the observations over several successive seasons. Failure to do this by earlier workers has resulted in somewhat incorrect notions regarding the disease.

II. Symptoms and Cause of the Disease.

The case of the disease which formed the starting-point of the present investigations was met with on a farm in Co. Dublin in the first week of August, 1909. The plants were of the variety Duchess of Cornwall (a type of Up to Date), and were being grown in a small plot.

The affected plants, which were distributed promiscuously through the plot, were of fair size, certainly not dwarfs, but not so large as their healthy neighbours. They were clearly distinguished from the healthy plants by the fact that their lower leaves were brown, shrivelled, and practically dead. The upper, younger internodes of the stalks had failed to elongate normally, and the leaves borne on them were more or less crowded together, forming a kind of rosette. The individual leaflets of these upper leaves were folded upwards and inwards on their midribs, consequently exposing their lighter-coloured under-surfaces to view.

On cutting the stalks transversely, the wood of three principal vascular bundles was seen to be discoloured, being of a yellowish brown tint and not so dark as is usually the case in attacks of Black Stalk Rot due to *Bacillus melanogenes*. On pulling the stalks from the ground, it was at once clear that the disease was not Black Stalk Rot, because the portions of them near the surface of the ground and below it were not black and rotten, but, externally at any rate, apparently quite healthy.

The parent tubers from which the diseased plants had sprung were, in the cases examined in detail, already rotten ; but it may be stated here that such tubers do not always decay in this manner, for very often they may be found, at the close of the season, hard and apparently sound.

Amongst the newly formed tubers rather small-sized ones predominated, although a few quite large ones were sometimes also present. The total yield from the diseased plants was considerably less than that from the neighbouring healthy ones. The tubers produced by the affected plants showed no external signs of any disease. When, however, these tubers were cut across at their heel (proximal) ends, most of them showed a brown, discoloured vascular ring, due, as subsequent microscopical examination showed, to the browning of the walls of the elements of the wood, and, in the region

quite close to the point of attachment of the rhizome, to discolouration of the walls of other cells also. (See fig. 7, Plate III.) This browning of the vascular ring could be seen with the naked eye, extending in some cases for quite a considerable distance towards the rose (distal) end of the tuber, and, with the microscope, it was still to be seen in sections of the vascular tissue made at the extreme rose end.

Transverse sections of the stalks of affected plants, made both in their over- and underground portions, and examined with the microscope, showed that the wood vessels were more or less thoroughly choked with branched, septate fungus mycelium which, in a rare instance or two, bore single-celled oval conidia within the cavities of the vessels. Similar mycelium was found in abundance in the wood vessels of the leaves, roots, and rhizomes of affected plants. From the rhizomes the mycelium was definitely traced into the wood vessels of the new tubers, and in one case, which will be described in detail later, it was followed by means of hand sections to a distance of four centimetres from the heel end of the tuber.

When cut portions of the affected plants (including tubers) were kept moist for a few days in covered dishes, the fungus grew out from the wood vessels and formed aerial, verticillately branched conidiophores, on the tip of each of which a glistening, spherical globule, containing a number of conidia, was borne. (See figs. 5 and 6, Plate III.)

When portions of affected stalks were allowed to remain for about twelve days under such conditions, they began to rot; and microscopical examination showed that the mycelium had spread from the vessels to the surrounding tissues, was turning black, and assuming a resting condition. (See fig. 4, Plate III.)

The fungus, which infection experiments have proved to be the cause of the disease, was identified as *Verticillium albo-atrum*, a species first described by Reinke and Berthold[1] in 1879, and assigned by them as the cause of a potato disease, which they regarded as Curl (Kräuselkrankheit).

The appearances described above are quite characteristic of the Verticillium disease. A study of it, however, over several seasons has shown that its external symptoms are subject to considerable variation in intensity. For instance, the curling or rolling of the leaflets has been found to be not an absolutely constant feature of the disease, and thus the terms "Curl" and "Leaf Roll" are to be avoided in speaking of this disease, the more so since they have been used in the past in a somewhat indiscriminate way, as will be seen from the discussion in a later section of this paper.

[1] Reinke, J., and G. Berthold, Die Zersetzung der Kartoffel durch Pilze. (Dritter Abschnitt. Die Kräuselkrankheit der Kartoffel.) Berlin, 1879, p. 67.

The view of the nature of the disease which has resulted from a study of it during several seasons is that it consists primarily of a more or less premature death of the plant owing to a gradual process of desiccation, proceeding from below upwards. It is to be regarded as a type of wilt-disease, although actual wilting of the foliage—*i.e.* a condition of flaccidity, limpness, or loss of turgor in the leaves while still green—is extremely rare in this country, and has only been seen in cases where healthy plants have been artificially inoculated through wounds with pure cultures of the fungus.

In cases of severe attack the affected plants may attain the height of only a few inches, and they may be killed off comparatively early and without developing any curl or roll in the foliage. On the other hand, in cases of very slight attack the plants may attain practically their normal size, and may not begin to die off much before the usual time. Rolling or curling of the foliage may here also be absent, and such cases of the disease are easily overlooked, particularly if the plants are at the same time attacked by blight (*Phytophthora infestans*) or by the Botrytis disease. Of course, the greater number of cases of the disease lie between these two extremes.

In many cases after the death of the plants black streaks are noticeable on the dead stalks. These are due to the production of the black form of mycelium by the fungus and must not be confounded with the black, adherent, flattened sclerotia of Botrytis so often seen on dead potato stalks.

The only certain means of diagnosing the disease is by finding the mycelium of *Verticillium albo-atrum* in the wood vessels of the plant or of the tuber from which it is derived. Even the absence of the mycelium from the wood vessels of the stem, root, &c., of a plant at a given moment is not necessarily proof that such plant is not diseased, or at any rate that it will not become so. For in many cases it has been found that the mycelium passes from the parent tuber into the stalk rather slowly, and the foliage may be just beginning to show typical symptoms of the disease before the mycelium has reached the stem. The non-realization of this possibility has doubtless been the cause of some at least of the contradictory statements made with regard to this disease.

Finally, it may be stated that no case of the disease has been met with in which the affected plant has failed to bear some new tubers; and since a large proportion of these tubers contain the fungus within them, the disease will spread to the next generation. The disease, therefore, is not extinguished automatically in any given generation of affected plants, as has been supposed.

III. Previous Investigation of the Disease.

As mentioned above, Reinke and Berthold in 1879 described the disease due to *Verticillium albo-atrum* under the name of Curl-Disease

(Kräuselkrankheit). When the present investigation was started, the account given by these authors was practically the only one dealing in detail with the Verticillium disease; and although during its progress some additional information concerning it has been published, such as that by Spieckermann and by Orton, which will be considered later on, yet Reinke and Berthold's work still remains the fundamental one on this disease. Before proceeding further, therefore, it will be necessary to give a brief résumé of the account of the disease as given by these authors, laying stress on those points which seemed to require further investigation before being accepted as conclusively established.

Reinke and Berthold describe the disease as making its appearance under three distinct forms or types (A, B, and C). Type A is seen towards the beginning of July, and is characterized by the wilting, drying up, and yellowing of the lower leaves, not accompanied, at least to any extent, by curling of the leaflets, or by brown spotting of the foliage. Fungus mycelium is present in the wood vessels situated in all parts of the plant. It is found in the new tubers, but is said never to proceed far into them, a distance of fifteen millimetres from the heel being the maximum observed. It is stated to pass the winter at the point of detachment of the tuber from its rhizome and partially within the tuber.

Type B of the disease is seen at the same time as type A. Some time after the middle of July, potato-stalks, which up to that time had appeared perfectly healthy, begin to show a curling of the leaflets at their edges, and brown spots begin to appear on the foliage. At length such spotted leaves become completely dried up. The tissues of all the overground portions of the affected stalks are completely free from fungus mycelium, but *in the cortex* of those portions of them situated below ground and in that of the roots it is stated that *Verticillium albo-atrum* is present. The fungus in this case also reaches the new tubers, but by exactly what route is not clear from the description. It is said to pass the winter as in the previous type at the very heel-end of the tuber, and it could never be traced any distance into the interior of the tuber.

Although the two types mentioned are generally well marked and were described separately, the authors admit that they occur not infrequently on different stalks of one and the same plant, and even sometimes on one and the same stalk. It is also admitted that in certain cases of type B of the disease, mycelium was found after a time in the vessels of the stalks. It may be stated at once that in the present author's opinion mycelium would probably always have been found in the wood vessels of all parts of plants affected with type B of the disease had the search for it not been made too early. Types A and B

of the disease then would appear to be essentially similar, the principal difference being that in type B the mycelium of the fungus takes somewhat longer to reach the plant from the parent tuber than is the case with type A.

Type C is stated to arise exclusively when tubers derived from plants affected with either type A or type B of the disease are planted, although such tubers may also give rise to "misses," *i.e.*, may produce no plants at all. Plants affected with type C of the disease come above ground late, develop slowly, and remain small. The leaves do not expand fully, and are not of the normal green colour. The leaflets are curled and wavy, the petioles being bent backwards. Death and desiccation of the plants take place from above downwards, and after the death of one stalk others may subsequently develop from the same tuber, but only to die away in their turn. The stalks are exceedingly brittle, and the plants are said to die without producing any new tubers, so that the disease exhausts itself in this, the second, generation.[1] No fungus mycelium is present in the vessels of plants of type C at any time; but the mycelium of *V. albo-atrum* is stated to be present in the cortical tissues of the subterranean portion of the stalks. The seed-tubers producing this type of the disease, when they have not already rotted in the ground, contain no mycelium in their internal tissues; but Verticillium is stated to be present in the cells of the skin, although it does not penetrate into the subperidermal tissues.

What strikes one as rather remarkable in Reinke and Berthold's account of the disease is the absence of the fungus from the wood vessels in types B and C, and, as has already been surmised, this may possibly be due to the fact that a long enough period was not allowed to elapse before the examination was made.

Much stress is laid on the point that the fungus is only present at the very heel-end of the tubers derived from plants affected with the A and B types of the disease, and that even at the end of the season when such tubers had produced plants of the C type mycelium could not be found in their interiors.

The mode of transmission of the fungus from the first generation (A and B types) to the second (C type) is, as described, a most peculiar one. It is stated that when the tubers having the mycelium strictly limited in location to their heel-ends are planted in the spring the mycelium grows *around the outside of the tuber* in the cork layers of the skin, and without penetrating the interior. Having in this way reached the bases of the young sprouts, it

[1] In a postscript it is stated that in a few cases tubers from plants affected with the A type of the disease gave rise to plants of the B and not of the C type; hence it is assumed that in such cases the disease would become exhausted in the third generation.

finds its way into them, probably through the cortical portions of the young roots. It was certainly to be expected that since the fungus was located primarily in the vessels of the affected stalks and entered the new tubers, it would pass in through the vessels, remain in them during the winter, and pass out from them into the vessels of the new stalks in the spring; but it is quite clear that Reinke and Berthold did not admit this view.

It was particularly with a view of throwing light on this point that the present investigation was started, although it was also desired to obtain information on other phases of the disease and on the behaviour of the fungus in pure culture. The account of the fresh investigations on the disease will, therefore, now be proceeded with; and the small amount of further literature on it, published while the investigations were proceeding, will be dealt with where necessary.

IV. Localization of the Mycelium in Affected Tubers.

It is a matter of no difficulty to trace by means of suitable sections the passage of the mycelium from affected stalks through the rhizomes and into the heel-ends of the new tubers. It passes exclusively through the wood vessels. This has been studied, not merely by sections, but by suitable incubation of portions of rhizomes as well as of tubers, proving that the fungus involved was the species of Verticillium in question.

All the tubers borne by an infected plant, however, do not necessarily become infected. As a rule the fungus only reaches the older and larger tubers, while the smaller and younger ones often remain free from invasion. Here it may be stated that the tubers which do not become infected with the mycelium of the fungus produce, when planted, absolutely healthy plants, and their progeny continues to do likewise. Hence, by separating out the non-infected tubers (by the method to be described subsequently) from a mixed stock of seed-tubers derived from affected plants, and by employing them as seed-tubers, it has been possible to raise a new stock free from the disease.

As has already been pointed out, Reinke and Berthold maintain that when the fungus reaches the heel-end of a new tuber it remains strictly localized there. Spieckermann[1] also states that at the time of digging, the mycelium is chiefly confined to the heel-end of the tuber, and seldom proceeds further or reaches the rose-end. He believes that during the winter resting period of the tuber the mycelium does not make any further progress, since even in spring its growth has not advanced, and the sprouts are, therefore, all viable.

[1] Spieckermann, A., Beiträge zur Kenntniss der Bakterien- ring- und Blattrollkrankheiten der Kartoffelpflanze. Jahresber. d. Ver. f. angew. Bot., viii, 1911, p. 1.

This author, however, does not provide any experimental evidence in favour of his contention; and the observations and experiments now to be described show that these views do not hold good.

In 1909 a large tuber, nearly thirteen centimetres long, borne by an affected plant, was subjected to microscopic examination by means of sections of the vascular tissues removed from it, at intervals, in such a way that the use of the tuber, or rather portions of it, as "seed" for the following season would not be interfered with. By this means the mycelium was traced definitely in the vessels of the tuber to a distance of 4 cm. from its heel-end, and the browning of the woody tissue was visible to the naked eye for a distance of about 1.5 cm. beyond this point. Owing to the comparatively small size of the vessels, and the difficulty of cutting hand-sections accurately transverse to their long axis in the rose-end region, it was not possible to demonstrate with certainty the presence of mycelium in them by this method; but it was noted that the walls of these vessels appeared, under the microscope, distinctly browned, although this was not evident to the naked eye.

This tuber was, in the autumn, cut transversely into two portions, the cut being made some distance nearer to the rose-end than the point to which the mycelium had definitely been traced. The heel-end portion had two eyes on it, while the rose had three. In the spring of 1910 the two halves were so cut that five sets were available, each containing one eye. These sets were planted in sterilised soil, each in a separate pot. Sets 1 and 2 were from the heel-half of the tuber in which the mycelium was known to be present, while Sets 3, 4, and 5 were from the rose-half in which the presence or absence of mycelium was doubtful in the autumn. It could not, at any rate, have reached them during the winter from the heel-portion of the tuber because this had already been cut off in the autumn.

The five sets were planted towards the end of April, and all of them except No. 3 produced small plants which up to the middle of July showed no symptoms of any disease. Set No. 3 was totally destroyed by a soft-rot apparently of bacterial origin, while the plant derived from Set No. 4 was practically destroyed by an attack of caterpillars. As time progressed little or no signs of rolling were observable in the leaflets of the plants; but the lower leaves began to wither and dry up; and before the end of August all three plants were practically dead, having succumbed to a progressive desiccation proceeding from below upwards. Microscopical and cultural examination showed that the vessels of these plants were completely choked with the mycelium of *Verticillium albo-atrum*, which was also present in the roots of the plant derived from set No. 4. In two cases (Nos. 1 and 5), in

which the old sets had not rotted, but were fairly well preserved, the fungus was found to be present in their now strongly browned woody tissues.

From this experiment it seems safe to conclude that the fungus was present in the autumn in the original tuber, not merely localized at the heel-end, but also at a distance from it at least greater than one-half of the length of the tuber.

The question of the exact location of the fungus within the tuber is an important one from the practical point of view. For, if it is strictly confined to a small area near the heel-end of the tuber in the autumn, and does not progress further during the winter, it should be possible to get rid of the disease by merely cutting off the comparatively small portions of the heel-ends of affected tubers before using them for seed. This point was, therefore, gone into in further detail.

A preliminary experiment was carried out in 1910 with tubers derived from affected plants. Small portions of the heel-ends were first cut off, in order to see whether the vascular ring was browned or not. The tubers were then divided into two groups: (*a*) those with no browning, hence apparently healthy; and (*b*) those showing browning, hence presumably diseased. The tubers in group (*b*) were then further subdivided. From the heel-ends of one half of them the tissues were cut away until the browning of the vascular ring was no longer visible to the naked eye, while to those of the other half nothing further was done. By this means the attempt was made to divide the tubers into the following three classes:—(1) healthy; (2) primarily diseased, but rendered healthy by cutting away the diseased tissue; (3) diseased.

The tubers were planted under field conditions at a time when it was unfortunately impossible, owing to the pressure of other work, to devote as much attention to the behaviour of the resulting plants individually as was desirable. Further, a severe attack of blight, combined with another of the sclerotium disease, seriously interfered with the success of the experiment. Nevertheless, taking as a criterion the amount of rolling in the leaflets exhibited before these diseases gained the upper hand, it was possible to see that the plants derived from the tubers of Class 3 (diseased) were decidedly the worst, while there was not any clear difference between those derived from the tubers of Classes 1 and 2. Plants with Verticillium in the vessels of their stems were found, however, in all three classes, but such plants did not appear to predominate in one class more than in the others.

The experiment, although leaving much to be desired, showed at least that no reliance could be placed on naked-eye examination of the cut ends of tubers as a means of discriminating between those containing the fungus and

those free from it, and proved that the cutting away of the heel-ends of the tubers was not sufficient to eliminate the disease with certainty.

It was clear that in any further experiments on these lines it would be necessary to test individually each tuber before using it to ascertain whether it contained the fungus or not, and the method of testing was as follows:—

The tubers were first thoroughly cleaned by careful scrubbing in plenty of running water, and then dried in a clean linen towel. They were purposely not treated with any disinfectant, lest some of it should be absorbed into the wound at the heel-end of the tuber caused by its severance from the rhizome, which might prevent the development of the fungus if present.

A small portion of the heel-end of the tuber, about a millimetre or so thick, was then cut in such a way that it remained hanging from the tuber by a small bit of skin, the two cut surfaces being of course exposed. Tubers prepared in this way were put into clean covered glass dishes the bottoms of which were lined with moist filter paper. After standing for two or three days at room temperature microscopic examination of the tubers was made.

Verticillium when present in the wood vessels of a tuber does not produce aerial growth and conidiophores at the cut surfaces with the luxuriance generally observed when pieces of affected stalks, rhizomes, or roots are suitably incubated. Nevertheless it does grow out in varying degrees, if present, and by microscopical examination one can decide whether a tuber possesses it or is free from it, although cases do arise occasionally where definite decision is not possible.

Examination was always carried out at three places—(1) the natural wound at the heel-end; (2) the cut ends of the vascular tissue of the small portion of the tuber nearly severed; (3) the cut ends of the vascular tissue of the remaining part of the tuber. Only when the fungus was present or absent at all of these three places simultaneously was the tuber regarded as infected or free from infection respectively for the purpose in view.

It may be stated that the mycelium can be traced definitely growing out of the browned xylem portions of the vascular ring of the tuber, and, indeed, the exit of an individual hypha from the cavity of a particular wood vessel has in many cases been traced, so that there can be no doubt as to the source from which the fungus comes. Occasionally the growth of other fungi makes certain determination practically impossible, and this is especially the case when *Hypochnus Solani* is present on the surface of the tuber under examination, for it rapidly produces a luxuriant growth at the expense of the cells killed by the process of cutting. Even in such cases, however, with some experience it is sometimes possible to discriminate between the growth of

Verticillium emanating from the wood vessels and the coarse mycelium of Hypochnus covering the cut surface.

In the autumn of 1914 twenty-five tubers which were proved conclusively to contain the fungus, by testing as above described, were selected. In the third week of October, i.e. soon after they were dug, these tubers were each cut into two portions, a heel portion and a rose portion, each possessing eyes, the heel portions being as a rule larger than the corresponding rose portions. The fifty sets thus obtained were then indelibly marked with Indian ink, so that they could not possibly become mixed. They were placed in a sprouting box and allowed to remain there over winter. In the spring all of them had produced normal sprouts from the various eyes.

In January, 1915, a further set of twenty-five tubers was selected which were found on testing as before to contain the fungus. These were also allowed to sprout in a box, which they did in normal fashion. In April, a day or two before planting, these tubers were each cut into two portions, roses and heels respectively. The tubers cut in the autumn and in the spring were all selected from the same lot, and were the produce of affected plants grown in 1914.

At planting time in 1915, therefore, there were sets from twenty-five autumn-cut tubers, and a similar number from spring-cut ones. Five pairs of each were grown in pots in Dublin in a cool greenhouse, while the remainder were planted at Clifden. No farmyard manure was used in either case. The resulting plants, some of which were diseased and some healthy, were carefully examined for the presence or absence of mycelium in their stalks. In the cases of the healthy plants, where no mycelium was found, they were allowed to grow until the close of the season, when they were dying naturally, so that the absence of mycelium was not due to the plants having been examined too early.

A summary of the results is given in the following table :—

No. and description of sets planted.	No. of plants developed.	No. of misses.	Diseased plants with Verticillium.	Healthy plants without Verticillium.	% diseased of those developed.
25 Autumn-cut. Heels.	23	2	21	2	91
25 do. Roses.	24	1	15	9	63
25 Spring-cut. Heels.	23	2	22	1	96
25 do. Roses.	25	0	19	6	76

With regard to the "misses," these were due in the two cases of autumn-cut heels to the sets succumbing apparently to a soft bacterial rot. In the spring-cut heels the miss in one case was evidently due to the attacks of slugs, and in the other a weak shoot had developed from the small set, which, however, had also been attacked and was not strong enough to reach above ground. Verticillium was found to be present in the vascular tissues of the remaining more or less sound portions of these sets in July. The miss in the case of the autumn-cut roses was due to the removal of the set, probably by a rat or a jackdaw, as both of these animals were observed at various times to be active in this manner on neighbouring plots. It is not believed that the presence of *Verticillium albo-atrum* in the sets was the cause of any of these misses. This point will be referred to again later in this paper.

It is scarcely necessary to go into the details of the development of the plants from these various sets; suffice it to say that many of them, from roses as well as heels, were small from the start and soon showed signs of the disease, dying away soon after the middle of July. Others were larger and lasted longer, while comparatively few lasted out the season. In all cases in the table where the plants are described as containing Verticillium, it should be understood that this was determined both by microscopic examination and by cultural methods. The same methods were applied and gave negative results in the cases of the plants described as being without Verticillium.

It will be seen, as was to be expected, that a very high percentage of the heel-end sets, both autumn-, and spring-cut, gave diseased plants; nevertheless a few of them gave healthy plants, from which it follows that an affected set or tuber, although it generally gives rise to a diseased plant, does not necessarily do so. Further evidence of this will be adduced presently. It may also fairly be concluded that even in the autumn the fungus must have reached in very many cases (sixty-three per cent. in this particular experiment) the rose-end half of the tuber, and that consequently it is not correct to regard the fungus as generally hibernating in a more or less strictly localized position at the very heel-end.

Further, since the percentage of diseased plants arising from the spring-cut rose sets is considerably higher than that of the corresponding autumn-cut ones, it may be concluded that the mycelium does make some progress from the heel towards the rose-end of an affected tuber during storage over winter.

The fungus has been isolated from the vascular tissues of various regions in tubers both before planting and also at the close of the season when they have produced diseased plants. (See fig. 1, Plate III.) It has never been observed to spread from the wood vessels to the surrounding tissues in the

tubers as it does in the stalks, and the black sclerotial form of the mycelium has not been found in the tubers. It is true that affected tubers, when kept unplanted till the late summer, often show, when cut across, a strong blackening in the region of the vascular ring; but microscopic examination shows that this blackening is not due to the presence of mycelium, but to some colouring matter produced in the cells in this region.

From what has been said, it seems clear that the mycelium of the fungus which is present in the tubers produced by affected plants—and very few of the tubers produced by such plants are free from it—is located in the wood vessels of the vascular tissues and is confined to them. But it is by no means necessarily localized at the heel-end of the tuber, and may probably spread slowly in the vessels of the tuber during winter storage. Judging from the varying amount of aerial growth produced at the cut surfaces of the vessels of affected tubers when suitably incubated, it seems likely that the amount of mycelium present in such tubers may vary considerably, and that thus one tuber may be more strongly infected than another.

It is quite conceivable—although it has not definitely been proved—that the strongly infected tubers give rise to plants which show the disease early and soon die off, and that the less strongly infected ones produce plants which attain more normal size, and which do not show symptoms of the disease until considerably later. Finally, the fact that affected tubers occasionally produce healthy plants may be explained by the original infection of the tuber being so slight that the fungus was unable to reach the plant developing from such a tuber before the conclusion of its growth.

V. Production of Diseased Plants from Affected Tubers.

According to Reinke and Berthold, when affected tubers are planted, the fungus grows over the outside of the tuber in the cork cells, and so reaches the young sprouts through the cortical portions of the young roots. This idea was based chiefly on the fact that Verticillium was found growing on the skin of the tubers as well as in the cortical portions of roots and young stems. It does not follow, however, that the Verticillium found in such situations was in reality *V. albo-atrum*, and I am of opinion that Reinke and Berthold were misled by an assumption of this kind. I have found two apparently new species of Verticillium (which will be described in detail in another paper) which occur on potato tubers, and which in the conidial form might easily be mistaken for *V. albo-atrum*, but which, when grown in pure culture, are clearly quite different from it and from each other. Neither of these species is capable of producing a disease of any kind in the potato; they are both pure saprophytes.

It is much more reasonable to suppose that the fungus present in the wood vessels of the tuber passes from them at some time or other directly into those of the developing sprouts; and although this passage has not been definitely traced while it is taking place, yet there is every reason to suppose that it does occur.

There would be considerable difficulty in following the course of the mycelium from tuber to developing stalk while this is actually taking place, particularly because the passage does not appear to occur until after the sprouted tuber has been planted, and the young shoots have made considerable growth.

During the several seasons over which this disease has been studied, no evidence has been obtained that the presence of the fungus in the tuber interferes with the production of sprouts; on the other hand, affected tubers have always been found to sprout normally, and to be indistinguishable from healthy ones in this respect.

According to Miss Dale[1] *Verticillium albo-atrum* is a cause of "blindness" (non-development of sprouts) in potatoes. It seems clear, however, from her description that this author was not really dealing with this species of Verticillium at all, for it never forms "small rounded segments containing numerous drops of oil," although one of the two new species referred to above does this. The species associated with "blindness" was found in the epidermal and cortical tissues, and not, apparently, in the wood vessels. Further, no infection experiments were made, and it is quite possible that the "blindness" was due to some other cause, while the Verticillium present was simply living saprophytically on the dead tissues.

The following observations show that the fungus in the wood vessels of the tuber does not pass at once into the developing sprouts. Seven tubers from affected plants were tested in September, 1914, and were found to contain mycelium of *V. albo-atrum*. They were allowed to sprout in a box during the winter—which they did normally—and remained unplanted till the third week in July, 1915. At this time the tubers were somewhat shrivelled owing to loss of water, and each had produced several sprouts of varying lengths from the eyes situated both near the rose and heel ends of the tubers. A further test was then made of the tubers, and it was found that the mycelium in their vessels was still living. All the sprouts of each tuber

[1] Dale, E., On the Cause of "Blindness" in Potato Tubers. Ann. Bot., vol. xxvi, 1912, p. 129. I cannot, however, follow Wollenweber, who says (Phytopathology, iii, 1913, p. 40) that Miss Dale's Verticillium is probably "*Periola tomentosa* (Fr.), Reinke and Berthold (1879) [*sic*] . . . only resembling Verticillium macroscopically." As far as I have seen it, Periola does not resemble a Verticillium even macroscopically.

were then subjected to careful microscopical and cultural examination, but in no single instance could the fungus be found present in a sprout. In one case it was found in the wood vessels of a tuber close to the point of origin of the sprouts, but even here the sprouts themselves were free from it.

The young shoots produced by affected tubers always appear healthy (although they may be small) when first they come above ground; and if they are examined microscopically at this stage, mycelium may not be found in their wood vessels. But if they are allowed to remain longer, sooner or later symptoms of disease will begin to develop in their foliage, such as rolling of the leaflets, or dying off of the lower leaves. When this occurs, or at any rate very soon afterwards, mycelium will be found in the wood vessels of the lower portions of the stalks at least, while often it has not yet reached the upper parts. This mycelium has been traced down the stalk, back to the point of insertion of the latter on the parent tuber, and finally into the wood vessels of the tuber itself at this point, thus permitting of no reasonable doubt but that it has grown from the wood vessels of the tuber into those of the stalk. No evidence was found of the presence of the fungus in the cortical tissues of these stalks, and consequently it could not have reached the wood vessels from this source. Hence it is believed that the mycelium reaches the young stalks, not by growth over the outside of the tuber, as Reinke and Berthold supposed, but by direct growth through the xylem system common to tuber and stalk.

There is, however, a distinct pause of longer or shorter duration before the mycelium proceeds from the tuber to the stalk, during which temporarily healthy stalks are developed from affected tubers. If this pause is sufficiently prolonged, either because the amount of mycelium in the tuber was originally very small, or its location was removed so far from the developing shoot that the latter could not be reached in time, then it is possible to understand how an affected tuber may give rise to a plant which remains healthy throughout the season, and which produces healthy progeny.

Statistics compiled for the seasons 1912–1915 show that when affected tubers are planted 96 per cent. of them give rise to diseased plants bearing further affected progeny, while the remaining 4 per cent. produce healthy plants with healthy progeny.

VI. The Fungus in Pure Cultures.

Since when placed under suitable conditions of moisture and temperature, the fungus present in infected tissue grows out into the surrounding air, and produces a plentiful crop of conidiophores and conidia, it is a com-

paratively easy matter to obtain it in pure culture. This has been done in several instances by planting out the conidia in gelatine media either directly or after a first transference to suitable slants in test-tubes, or to media previously allowed to set in Petri dishes. In this last-named way it is possible to exercise microscopic control over the growth which develops from the inoculating material; and platings of conidia from growths of this kind, which are found to be free from bacterial or other contamination, have always given pure cultures. In some instances the additional precaution was taken of making successive platings before arriving at the particular culture destined to serve as a stock from which cultural and other studies were to be made.

The fungus has also been isolated from the vascular tissue of affected tubers both before planting and after they have been planted and have given rise to diseased plants. It frequently happens that the parent tubers from which diseased plants are developed are to be found at the close of the season more or less completely hard, sound, and not fully depleted of their reserve food materials. In such tubers the woody tissues containing the fungus are strongly browned, as shown in fig. 3, Plate III. Cultures from tubers were obtained by transferring—under conditions of asepsis—portions of the affected woody tissue direct to suitable media in slants or on set plates. Some of these direct transfers proved to be pure from the start, while others of course were contaminated by the presence of other organisms, especially in the case of old tubers; but even in these latter instances *Verticillium albo-atrum* was always present, and by further work could be obtained in pure culture.

The fungus grows well as a saprophyte on a variety of media such as Quaker Oat agar, oat-extract agar, wort gelatine, cooked potato agar, potato-stalk agar, cooked potatoes, cooked potato-stalks, and beef extract agar and gelatine.

It produces both aerial mycelium bearing conidiophores with conidia and submerged mycelium. There has been a tendency during the period of over two years through which the cultures have continuously been propagated for the relative proportion of aerial mycelium developed to diminish. Both the aerial and the submerged mycelium is at first pure white in diffused light, but in the course of time black submerged mycelium is produced just in the same way as occurs in the stalks of affected plants, and the medium therefore becomes blackened. This blackening is due solely and entirely to the blackening of the mycelium, and not to the production of dark chlamydo-spores, as occurs in the two other species of Verticillium already alluded to.

On certain media, viz., beef extract agar and gelatine and potato-stalk extract gelatine, this blackening of the submerged mycelium does not occur at all. Further, although the fungus readily develops this black submerged mycelium (except on the media just mentioned) when cultivated soon after its isolation from the potato, yet in the course of time, after prolonged cultivation on these media, its power of producing black submerged mycelium gradually diminishes until it becomes entirely lost.

Most of the cultures were kept in the diffused light of the laboratory, but a special comparative series was made to see whether light exercised any influence on the behaviour of the fungus. Parallel cultures were for this purpose (*a*) kept in total darkness, and (*b*) exposed directly to daylight in a window facing north but protected from direct sunlight. No very striking differences were observed in the cultures, but in the dark the aerial growth was pure white, while in the light it took on a faint pink fringe, and the blackening of the submerged mycelium which occurred both in the dark and in the light began to be perceptible in the cultures kept in the dark slightly sooner than in those exposed to light. On all the gelatine media used liquefaction of the gelatine invariably occurred. This took place perhaps somewhat slowly; but in slants in test-tubes the medium was usually completely liquefied in cultures at room-temperature one week old.

Many attempts were made to cultivate the fungus on living potato tubers and on cut portions of living green potato-stalks, but without success. A limited amount of development occurred in the cells injured by cutting or making the inoculation. In the case of the cut stalks further growth took place only when they had been standing for such a long time that they had begun to die. In the case of the tubers or cut portions of them no further growth took place, and a layer of cork was developed beneath the wounded surface. Hence, although the fungus is a vascular parasite of the potato, it is quite incapable of causing a rot of the living tubers or stalks.[1]

The morphological characters of the fungus were accurately described and figured by Reinke and Berthold, and its growth in pure culture has not resulted in the necessity of altering or adding to the description given by them. It is believed, however, that the species which these authors met with living on the skin of the tuber and in the cortex of the stalks below ground was not the same as that inhabiting the wood-vessels, but probably one of the two already referred to, which develop dark-coloured chlamydospores on their submerged mycelium.

[1] The opposite results obtained by Carpenter (Journ. Agric. Research V, No. 5, 1915, p. 203) are, as he himself points out, doubtful, and were probably due to imperfect experimental methods.

In spite of prolonged culture on varied media, *Verticillium albo-atrum* has not been found to produce any other reproductive bodies such as perithecia, nor has it ever produced chlamydospores of any kind.

VII. Infection Experiments.

The first attempt at producing the disease by suitable inoculation of a healthy plant was made at Clifden in the summer of 1909. The inoculation was made by inserting a portion of the woody tissue containing the fungus, obtained from a diseased plant, into a freshly prepared wound made to the depth of the wood just above ground-level in the stalk of a healthy plant, taking precautions to avoid, as far as possible, contamination with other micro-organisms. After nineteen days there were no distinct outward signs that infection had occurred; but as the foliage of the plant was beginning to be destroyed with the ordinary blight, it was decided to break off the experiment at this point. Sections of the inoculated stem showed the presence of fungus mycelium in the wood vessels to a distance up the stem of twenty centimetres from the wound; and on suitable incubation it proved to be that of Verticillium. Hence it appeared probable that there would be no difficulty in transferring the disease by inoculation.

In the foregoing case of course a pure culture was not employed, but in 1914 several series of infection experiments were made with pure cultures. Those of the first series were carried out on plants in pots in the greenhouse of the Seeds and Plant Disease Division of the Department of Agriculture in Dublin, and were as follows:—

The plants for the experiments were grown from previously sprouted healthy tubers from which all the eyes except the strong terminal one were excised. This was done in order that each plant might have but one substantial sprout or stalk suitable for inoculation. Inoculation was made in all cases by introducing portion of a pure culture into wounds carefully made in the sprouts or stems at a node, precautions being taken to prevent contamination by other micro-organisms. After inoculation the wounds were carefully covered with tinfoil, and were thus not allowed to come into direct contact with the soil. In every case where an inoculation was made a similar sprouted tuber or plant was treated in the same way, except that no portion of a culture was introduced into the wound; and this served as a control. In this particular series there were eight plants inoculated at four different periods, together with their eight controls. Some of them were inoculated in the sprouts before planting, others when the shoots were only about two inches above the soil, and the remainder at correspondingly later dates. Symptoms

of disease began to appear in the earlier inoculated plants about one month after inoculation; but in those inoculated later, when the plants were much larger, the symptoms first appeared within about seven days. The symptoms were the appearance on the older leaves of pale green or yellow areas with rather ill-defined margins. After a time these areas dried up and became brown and dead. Gradually these symptoms repeated themselves in the successively younger leaves, often but not always accompanied by an inrolling of the margins of their leaflets. In some cases the leaves while still green showed a true wilting due to loss of turgor in their cells; and by degrees the foliage of the inoculated plants died off by a process of desiccation proceeding from below upwards. When the foliage was dead, a similar process of desiccation took place in the stalks proceeding from above downwards. When the inoculations were made while the plants were still young, the inoculated plants remained much smaller than the corresponding controls; but this difference, although present, was not so marked in the case of plants inoculated when already fairly advanced in growth. A good idea of the kind of result obtained in this series of inoculation experiments is afforded by figs. 3 and 4, Plate II.

When the discolouring leaves showing the first symptoms of disease in the plant were removed and placed in a moist atmosphere, they quickly became covered externally with an aerial development of conidiophores of Verticillium, thus proving that the fungus had already reached them. Sections of the veins and petioles of such leaves showed the abundant presence of the mycelium in the wood vessels. The same mycelium was also found to be present in the stems, roots, and some of the new tubers of the inoculated plants at the conclusion of the experiment. It was an easy matter to obtain the fungus in culture from dead or dying plants, and to prove by the character of the black submerged mycelium developed in due time that it was *Verticillium albo-atrum.*

The control plants were just as carefully watched and examined for the presence of the fungus as the inoculated ones, but they showed no signs whatever of disease, and no mycelium could be found in their vessels; in short, they remained perfectly sound, and produced sound tubers.

The second series of inoculation experiments was carried out in one of the gardens at the Albert Agricultural College, Glasnevin, in the open ground. Six previously sprouted healthy tubers were inoculated in the sprouts before planting, and six plants from healthy tubers were inoculated in their stalks below ground in the manner previously described, when they were about 30 cm. high. There were twelve controls. All of the inoculated plants remained smaller than the corresponding controls. They soon began to show

the characteristic symptoms of the disease, and they died off prematurely, whereas the controls behaved normally. Microscopic examination of the inoculated plants showed the fungus present in all their organs, including some of the new tubers. Similar examination of the controls showed that ten of them were quite free from the fungus. In the case of one of the remaining two, it was found in the vessels of a single rhizome, but in no other portion of the plant, and in the case of the other it was present in the vessels of some of the roots. These two plants were growing in close proximity to two of the inoculated plants, and it is quite possible that the rhizome and the roots may have become infected by coming into contact with the diseased roots or rhizomes of these neighbouring plants; it would have been better if the controls had been planted at a greater distance from the inoculated plants.

A further series of inoculation experiments was carried out in the field at Clifden, there being four inoculated plants and four controls. Two of these and the corresponding controls were on plants of the variety Up-to-Date, which was the one used in the previously described series. The controls remained perfectly sound, while the inoculated plants became diseased, and were found to contain the fungus in all their parts, as in the cases already described.

The other two inoculated plants and the corresponding controls at Clifden were, however, of the varieties Shamrock and Northern Invincible respectively. Both of these varieties have proved themselves to be resistant to the attacks of blight (*Phytophthora infestans*), the former very highly so and the latter slightly less highly. The method of inoculation was the same as that employed in the previous experiments. The plant of Northern Invincible became diseased, and invaded in all its parts by the fungus, just as had been the case with the Up-to-Dates, while some of the tubers also contained it. On the other hand, the symptoms of disease in the inoculated plant of Shamrock were less pronounced, and the invasion by mycelium was much less extensive. The fungus was found in the vessels of the upper portions of the inoculated stem; but just below the inoculating wound it stopped, and it did not proceed further downwards. The roots, rhizomes, and tubers were found to be free from it. Hence it would appear probable that this variety is somewhat resistant to this disease, as well as to the blight. The controls in this case also remained sound, and were found to be free from mycelium.

Still further inoculation experiments were carried out on cut stalks placed in Tollens' nutrient solution, and kept under observation in the laboratory. It is not necessary to deal with these in detail; suffice it to say that the fungus spread from the inoculating wound in the vessels, both up and

down the stalk, but in a somewhat less vigorous fashion than where the stalks remained on the plant.

Some of the tubers produced by inoculated plants in 1914, which investigation showed contained the mycelium of the fungus in their vascular tissues, were planted in 1915, and gave rise to diseased plants, which could not be distinguished from similar plants arising from infected tubers produced in the previous season by naturally diseased plants.

Berthold and Reinke carried out infection experiments with the fungus, but, of course, not in pure culture. A considerable number of them gave negative results, particularly when the inoculations were made only into the parenchymatous tissue of tubers, or that of the cortex of stems. Positive results were, however, obtained, when the inoculation wound included a portion of the woody tissue, and the inoculated plants showed their type A of the disease.

The experiments described conclusively show that *Verticillium albo-atrum* is an active parasite capable of luxuriant growth and development in the larger vessels of the woody tissue of the potato, and that it is the direct cause of the disease described.

In the cases of the disease studied by the present author the source of the infection of the plant has generally been due to the planting of affected seed-potatoes, and no experiments have yet been carried out to ascertain the way in which primary infection of plants derived from healthy tubers might occur under natural conditions. Reinke and Berthold showed that when a young root was brought into contact with the mycelium of the fungus, the latter penetrated into the superficial tissues of the root, but apparently it did not reach the vascular tissues. Infection experiments with conidia placed on the surfaces of young roots did not succeed, as the conidia did not germinate. It seems probable that primary infection may occur directly from the fungus living as a saprophyte in the soil; but further experiments in this direction with pure cultures of the fungus are necessary before a definite conclusion can be drawn. Miss Dale[1] claims to have isolated *Verticillium albo-atrum* from a sandy soil, and cultivated it on various media. Since it is stated that the fungus in all its stages is a pure white, it might seem improbable that it was in reality *V. albo-atrum*. Nevertheless, in view of the fact that the fungus when grown for a long period as a saprophyte gradually loses its power of producing black mycelium, it is possible that the same loss had occurred owing to a prolonged period of saprophytic growth in the soil. In a case of

[1] Dale, E., On the Fungi of the Soil. I. Sandy Soil. Annales Mycologici, x. No. 5, 1912, p. 465.

this kind, suitable inoculation experiments with potato plants would enable a definite decision to be made.

VIII. Relation of the Verticillium Disease to "Curl" and "Leaf Roll."

The term "Curl" has long been in use for certain potato troubles. A disease known by this name is said to have been seen first in England so long ago as 1764; and towards the end of the eighteenth and during the early part of the nineteenth centuries it was the subject of much discussion by agricultural writers of the day. The literature on the subject is very extensive, but, unfortunately, in the main it is most unprofitable reading. From a study of this literature it is impossible to obtain an accurate idea of what the Curl really was, for most of the writers on the subject preferred exercising their imaginations in speculations as to the probable causes of the disease and in formulating remedies for it, rather than attempting to give a careful description of the symptoms which characterized it. It is quite possible, and indeed probable, that the term "Curl" was applied to more than one distinct disease of the potato; and this supposition, if correct, would serve to explain the conflicting opinions often published concerning it. Anyone who wishes to get an insight into the complexity of the views concerning "Curl" which prevailed during the early part of last century cannot do better than consult such a summary of them as that published in 1804 by Forsyth.[1]

Apparently as time went on the trouble known as "Curl" became less serious, and in the middle of the nineteenth century it was for a time quite overshadowed in importance by the epidemics of blight from which the potato crop then suffered. Nevertheless, it is clear from Hallier's[2] account that the disease (Kräuselkrankheit) was still prevalent in the early seventies of last century in many districts in Germany. Hallier's description of it would lead one to believe that it was one caused by a specific fungus inhabiting the vessels of the wood, and perhaps identical with the Verticillium disease described in the present paper. He himself certainly adopted this view, and named the supposed fungus *Rhizoctonia tabifica*. It is clear, however, that Hallier applied this name not to a single fungus, but to more

[1] "The Principles and Practice of Agriculture," systematically explained, in two volumes; being a treatise compiled for the fourth edition of the Encyclopædia Britannica, and revised and enlarged by Robert Forsyth, Esq. Edinburgh: Constable & Co., 1804. See particularly p. 174 et seq.

[2] Hallier, E., "Die Ursache der Kräuselkrankheit." Zeitsch. f. Parasitenkunde, iv, 1875, p. 97.

than one of those which are frequently found growing together on dead or dying potato-stalks.[1] The principal literature on Curl (Kräuselkrankheit), from Kühn's time onward, is dealt with by Reinke and Berthold in their paper already referred to, so that it is not necessary to go further into the matter here. It seems fairly clear that what is in the present paper called the Verticillium disease was sometimes included in what was called Curl (Kräuselkrankheit) in the seventies of the nineteenth century. Whether, however, the disease called Curl in the previous century applied solely to the Verticillium disease is another question. On the whole, it seems likely that it was only one of the diseases covered by this term.

There is another form of "Curl" to which I called attention in 1912,[2] which cannot well be confounded with the Verticillium disease, and the cause of which is quite unknown. Affected plants are small, and their foliage is very much crumpled and curled. No fungus is present in the plants; they produce few and only small tubers, which reproduce the disease when planted. In the cases studied by me the tubers produced in successive seasons became smaller and smaller until, finally, they were not large enough to remain alive until the spring, so that the race of abnormal plants died out completely. This form of Curl has also been recognized in Germany and in America, the term "curly dwarf" having recently been applied to it in the latter country.

Again, in England the term "leaf-curl" has recently been applied[3] to a potato disease said to be due to *Macrosporium solani* (Cooke), but which still lacks proper scientific study and investigation. Further, Vañha,[4] in 1910, regarded a new fungus (*Solanella rosea*) as being responsible for a potato disease, which he designated "curl" or "roll" disease (Kräusel- oder Rollkrankheit), without, however, bringing forward really convincing evidence.

Without going any further into the matter, it is quite clear that the term "Curl" (or "Leaf-Curl") has been applied to several probably distinct diseases of the potato, and it would therefore be well to refrain from the use of it in future.

In 1907 Appel[5] published a leaflet describing a disease of the potato which

[1] The sclerotium, with appendages, described and figured by Hallier as a resting stage of his *R. tabifica*, is, I have discovered, an early stage in the formation of its fructification by a species of Colletotrichum, which I hope to describe elsewhere.

[2] "Jour. Dep. Agric. and Tech. Instr. Ireland," xii, 1912, p. 354.

[3] "Journal Board of Agric.," vol. xii, 1905–1906, p. 476. Ib., vol. xiii, 1907–1908, p. 466.

[4] Vañha, J., "Die Kräusel- oder Rollkrankheit der Kartoffel, ihre Ursache und Bekämpfung." Monatshefte f. Landwirtschaft, iii, 1910, p. 268.

[5] Appel, O., "Die Blattrollkrankheit der Kartoffel." Kais. Biol. Anst. f. Land- u. Forstwirtschaft. Flugblatt 42, 1907.

was then prevalent in Germany and some of the neighbouring countries. The disease was stated to be by no means a new one, but rather one which had hitherto been included amongst that congeries of diseases known as "Curl" (Kräuselkrankheit). To this particular form of disease the name Blattrollkrankheit ("Leaf-Roll" disease) was given, and one or more fungi (not specifically named) belonging to the genus Fusarium were said to be the cause of it.

This publication may be looked upon as the first of a long series of others which followed dealing with "Leaf-Roll." In some of these Appel's results are confirmed, and to some extent amplified. Thus, not only was a Fusarium found to be the cause of the disease, but in many cases the parasite was found to be *Verticillium albo-atrum*.[1] On the other hand, many students of the "Leaf-Roll" disease stoutly maintained that it was not due to a fungus at all, for the simple reason that the presence of a fungus in the vessels (as described by Appel) was by no means a constant or, in some cases, even frequent symptom of the disease. Thus arose a controversy between those who regarded this disease as of parasitic origin and those who maintained the contrary, and who explained it as being due perhaps to the upsetting of enzymic equilibrium, or some other such occult cause. It is not necessary to deal here with the literature which this controversy brought into existence; suffice it to say that had the upholders of the non-fungus theory of the cause of "Leaf-Roll" been acute enough to realize that the disease with which they were dealing was not the "Blattrollkrankheit" as first defined by Appel at all, but something quite different, the controversy would probably never have arisen. However, the development of this controversy need not be regarded as having served no useful purpose, for it has certainly been the means of stimulating research into some of the more obscure diseases of the potato, from which good results have followed.

It will be seen then that just as the term "Curl" is not applicable strictly to any one specific disease, so the term "Leaf-Roll," although introduced to apply to the specific disease caused by species of Fusarium invading the wood vessels, came to connote not only this disease, but also a similar one due to *Verticillium albo-atrum*, and, further, another (or possibly others, for the matter cannot yet be regarded as definitely settled), the cause of which is still not sufficiently well understood.

A distinct step forward in the direction of a clearing up of the prevalent confused ideas concerning Leaf-Roll was the publication in 1914 of a

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[1] Appel, O., "Einiges über die Blattrollkrankheit der Kartoffel." Jahresber. Ver. Angew. Bot., vi, 1909, p. 259.

Bulletin compiled by Orton.[1] Here for the first time, apparently, the disease due to Verticillium is distinctly dissociated from Curl and Leaf-Roll diseases, and placed in the group of Wilt diseases along with one previously known in America from the work of Smith and Swingle, and stated to be caused by *Fusarium oxysporum*. It follows that Appel's Blattrollkrankheit as originally described by him would also logically find a place in this group, although the actual species of Fusarium causing it may not be identical with that found in America, and even although the external symptoms of wilting, &c., were not absolutely identical in the two continents. All these diseases have this in common, viz., that more or less premature death by desiccation of the plant occurs owing to the choking of the wood vessels with fungus mycelium. It has been proposed to apply the term *vascular-mycosis* to diseases of this type; but the term is not above criticism. Since the fungus mycelium is, at any rate in the early stages of the disease, confined to the wood vessels, and since these form only a part of the vascular system of a plant, it would seem much more desirable to use a still more precise term, and I, therefore, suggest the word "*hadromycosis*" for use in this connexion. It must be remembered that the vascular system of a plant does not consist of xylem alone; and, in view of the fact that another disease, which will be referred to in a moment, has been designated "*phloem-necrosis*," it seems eminently desirable to employ the term "hadromycosis" instead of the more clumsy one "vascular-mycosis."

It is interesting to note that Appel in one of his most recent publications[2] proposes to follow Orton, and says, "I wish to eliminate the name leaf-roll disease for the parasitic troubles, and substitute the name vascular-mycosis."

The term "Leaf-Roll" or "Blattrollkrankheit" should, therefore, now be applied only to certain diseases of the potato showing rolling of the foliage in which parasitic organisms are, as far as is known at present, not the causative agents. Whether there is only one such disease, or whether the term may still, for the present at any rate include several diseases, cannot be decided until further research throws the necessary light on the matter.

Very important work on Leaf-Roll (using the term in its restricted sense as above described) has recently been done by Quanjer.[3] This author

[1] Orton, W. A., Potato Wilt, Leaf Roll, and related Diseases. Bull. 64. U. S. Dept. of Agric. Feb. 1914. [To this Bulletin is appended a very useful Bibliography of the subject under discussion.]

[2] Appel, O., Leaf Roll Diseases of the Potato. Phytopathology, vol. v., No. 3, 1915, p. 139.

[3] Quanjer, H. M., Die Nekrose des Phloëms der Kartoffelflanze, die Ursache der Blattrollkrankheit. Med. v. d. Rijks Hoogere Land-, Tuin-, en Boschbouwschool, vi, 1913.

shows that the disease is characterized by the degeneration of the phloem elements in the vascular tissue of affected plants, and he, therefore, uses the term "phloem-necrosis" to describe it. What the exact cause of this lepto-necrosis (as it might also possibly be termed) may be is not yet understood; but further investigation will, no doubt, yield interesting and possibly far-reaching results.

The conclusion as regards the Verticillium disease is, however, a clear one, viz., that it is a specific disease caused by a definite parasitic fungus, and, as such, it is not for the future to be classed under either of the somewhat indefinite terms "Curl" or "Leaf-Roll," but rather to be regarded as a specific type of hadromycosis.

IX. Distribution of the Disease, Losses caused by it, and Suggested Preventive Measures.

The Verticillium disease of the potato seems to be fairly widespread on the continent of Europe, and is present in the United States of America. It is probably more or less prevalent in Great Britain, although, so far as I am aware, no British mycologist has reported its presence there.[1] Marshall Ward[2] published many years ago a preliminary description of a disease which very possibly may have been this particular one; but apparently the matter was never thoroughly investigated. Orton,[3] however, definitely states that he obtained specimens of the disease in England from Ormskirk and Reading.

Until the season of 1915, when two other cases of it came under my notice, both of them in early potatoes of the variety Beauty of Hebron, the only case of this disease which I had observed was the one found in 1909 and propagated ever since then at the Clifden Investigation Station for purposes of study. It should be stated that no special means have been taken to ascertain to what extent exactly it may be prevalent in Ireland, and it may possibly be commoner than one supposes. On the other hand, if it were really common, and if it caused appreciable losses, it is most unlikely that its presence in this country would have escaped the attention of the very numerous agricultural Inspectors, Instructors, and Overseers who are in such constant and close touch with our farmers.

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[1] As has already been pointed out, the species mentioned in Miss Dale's paper was probably not *Verticillium albo-atrum*, but some allied species.

[2] Ward, H. M., A Potato Disease. Rep. British Assoc. for Adv. of Science, 1898, p. 1046.

[3] *loc. cit.*, p. 87.

As regards losses, these are not easy to estimate, but for the whole country they cannot at present be considered serious. Weighings were made in 1914 of the yields of diseased and healthy plants growing under similar conditions of cultivation on one and the same type of soil. The average yield per plant of forty-seven healthy plants was thirty-six ounces, while that of forty-two diseased plants was only twenty-seven ounces, so that there was a loss of one-quarter of the crop owing to the disease. The plants were growing in both cases in very poor soil, and in the absence of the usual dressing of farm-yard manure; and it is quite possible that in a rich and well-manured soil the difference in the yields between healthy and diseased plants might be still more pronounced. Hence if the disease by any means became widespread throughout the country, the losses might be very considerable.

Since the disease is in all probability primarily contracted from the soil, a proper course of rotation of crops should be followed, and potatoes should not be cultivated for successive seasons on the same land. Where a diseased crop has been grown the soil is almost certain to be contaminated thereby, owing to the decayed and infected portions of the roots, rhizomes, and stalks remaining in it after the crop is lifted. These portions of the plant contain the fungus in them in its resting form ready to develop when the conditions are favourable for it. It is a sound practice to burn potato-stalks when the crop is lifted, as they may harbour not only the Verticillium disease, but also other pests.

The chief danger of introducing the disease into a new locality lies in the use of infected seed-tubers. Growers of potatoes for seed purposes should be particularly on the alert with regard to this disease. Should any affected plants be observed in the crop, it would be best that the crop in such a case should not be used for seed at all. If the affected plants are but few and far between, each one should be dug out lock, stock, and barrel as soon as possible. The tubers might be cooked and fed to pigs, but the foliage, stalks, and roots should be destroyed, as far as possible, by burning. Should growers have any difficulty in recognizing the disease, they should avail themselves of the advice of technical experts, which is now so easily obtainable. Farmers should scrutinize their seed-potatoes closely, and should look with suspicion on any tubers which show a brown discolouration of the vascular ring when cut across near the heel-end. Tubers showing this brown ring are not necessarily diseased, but very frequently they are, and they should be submitted for examination by a competent authority before being purchased or used for seed purposes.

It has been shown that all of the tubers produced by a diseased plant are

not necessarily infected, although the great majority of them certainly are. It has also been found that a small percentage of affected tubers may produce healthy plants with sound progeny, so that with proper care it would be possible in the course of a couple of seasons or so to raise a fresh stock of healthy seed-tubers from a previously diseased one.

It might be possible also to heat affected seed-tubers to a temperature high enough to kill the fungus in the wood vessels without injuring the vitality of the tubers themselves, as can be done with tubers attacked with *Phytophthora infestans*; experiments on this point are now being carried out, but the results will not be known for some time.

X. Summary.

The Verticillium disease of the potato is one which results in the more or less premature death of the plant, the general symptoms exhibited being those of a process of gradual desiccation.

The mycelium of the fungus *Verticillium albo-atrum* R. and B. is found in the wood vessels of all parts of affected plants. It passes into the wood vessels of the new tubers, and from these again, in the great majority of cases, into the plants which develop from them. Hence the disease is transmitted by means of infected tubers. The fungus in the tuber is not necessarily strictly localized at or near the heel-end, as previous authors have supposed.

The fungus grows well in pure culture as a saprophyte, and infection experiments on healthy plants carried out with pure cultures were successful in reproducing the disease.

The disease was, to some extent at least, formerly covered by the terms "Curl" and "Leaf-Roll," but it is now to be removed from this category, and to be regarded as a specific type of those diseases in which the wood vessels become infested with fungus mycelium and for which the general term *hadromycosis* is suggested.

The disease does not appear to be very common in the British Isles, and the losses due to it are at present probably not large; but should it become prevalent, the losses might be severe. The most satisfactory preventive measures are to maintain a proper rotation of crops, and to take steps to ensure that the potatoes used for seed purposes are healthy.

XI. EXPLANATION OF PLATES.

PLATE II.

Fig

1. A potato plant derived from a tuber naturally infected with *Verticillium albo-atrum*, and showing in the upper leaves of the central stalk the first external symptoms of the disease. Photographed August 12th, 1913.

2. The same plant completely killed as a result of the attack. Photographed September 17th, 1913.

3. The plant on the left (*a*) was inoculated in a sprout on May 11th, 1914, before planting, with a pure culture of *V. albo-atrum*. The photograph was taken on July 14th, 1914. The gradual and premature death of the foliage proceeding from below upwards will be observed. The control plant (*b*) was not inoculated, and was perfectly healthy.

4. The same two plants photographed six days later when the inoculated plant was quite dead, but the control still healthy.

PLATE III.

1. The mycelium of *V. albo-atrum* developing at the cut surfaces of wood vessels in the vascular ring of an old tuber which had produced a diseased plant. × 50.

2. The black mycelium of *V. albo-atrum* developed in pure culture. No chlamydospores are present. × 220.

3. Portion of a tuber which had produced a diseased plant, with the cortical tissues removed and showing the strongly browned network of woody tissue which still contained the mycelium of the fungus. × 1⅓.

4. Longitudinal section through the xylem of an affected potato-stalk showing the blackened resting mycelium in the vessels and adjacent elements of the wood. × 224.

5. Conidiophores of *V. albo-atrum* showing the spheres of conidia on the tips of the verticillate branches. × 60.

6. Cut portions of affected potato-stalks which had been kept moist for a few days, showing the tufts of mycelium of *V. albo-atrum* growing out at the cut ends of the wood vessels.

7 An affected tuber with a thin slice of its heel-end removed, showing the discolouration of the tissues due to the presence of *V. albo-atrum.*

1.

2.

a *b*

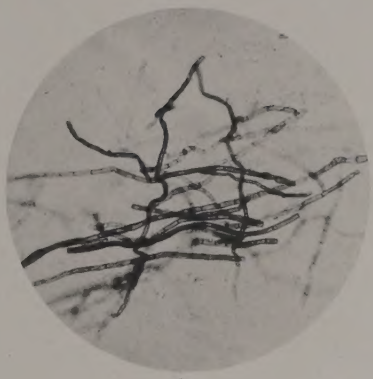
3.

a *b*

4.

M'Farlane & Erskine, Edinburgh.

1.



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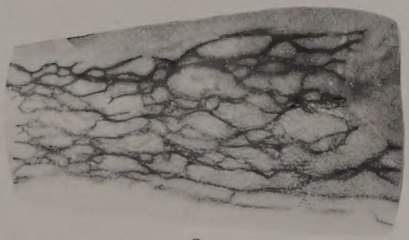

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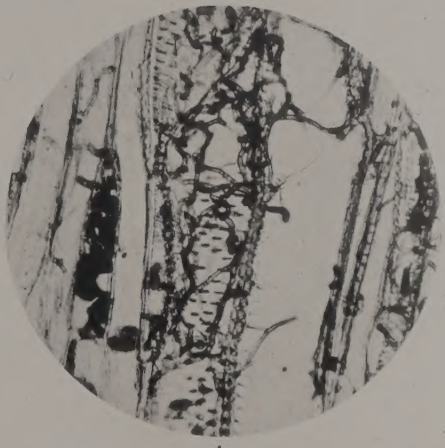

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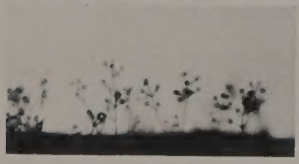

5.

6.

7.

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